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The pathogenesis of necrotic proliferative colitis in swine is linked to whipworm induced suppression of mucosal immunity to resident bacteria

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Abstract

Mucohemorrhagic enteritis syndrome in swine has a complex etiology with largely unknown pathogenesis. We have observed that inoculation of pigs with swine whipworm, *Trichuris suis*, initiates an interaction with resident bacterial flora to induce mucohemorrhagic enteritis. The role of bacteria in this mixed infection was demonstrated using 4 treatment groups. One group of pigs was inoculated with 2500 embryonated *T. suis* eggs alone, while a second group received *T. suis* eggs along with broad spectrum antibiotic treatment. Two other control groups of pigs were uninoculated and were either treated with antibiotic or untreated. Pigs inoculated with *T. suis* eggs exhibited diarrhea, mucosal edema, inflammatory cell infiltration, bacterial accumulation at the site of worm attachment in the proximal colon, and intestinal adenomatosis associated with the intracellular Ileal symbiont *intracellularis* bacteria. In addition, enlarged lymphoglandular complexes (LGCs) containing numerous extracellular bacteria, eosinophils, lymphocytes, macrophages, and neutrophils were observed in the distal colon. The other group of pigs that was inoculated with *T. suis* but treated with antibiotics had lesions localized to the site of worm attachment and histologically normal LGCs with no invasive bacteria in the distal colon. The groups of uninoculated pigs, with or without antibiotic treatment, exhibited no pathology or bacterial invasion. It appears that the complex pathogenesis of necrotic proliferative colitis in pigs may be linked to worm induced suppression of mucosal immunity to resident bacteria. Further, the association between bacteria, lymphocytes and macrophages in the LGCs of pigs infected with *T.*

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suis suggests an antigen-processing role for these structures in the colon. Further, the complex pathogenesis of necrotic proliferative colitis in pigs may be linked to worm induced suppression of mucosal immunity to resident bacteria.

Keywords: Swine diarrhea; *Trichuris suis*; *Campylobacter jejuni*; Ileal symbiont *intracellularis*; Mucohemorrhagic enteritis; Lymphoglandular complexes

1. Introduction

Mucohemorrhagic diarrhea is frequently reported as a cause of clinical signs and economic losses in weaned swine (Tubbs, 1987; Bliss, 1991). The etiology and pathogenesis of mucohemorrhagic diarrhea is complex. Bacteria like *Serpulina hyodysenteriae*, *Salmonella choleraesuis*, *Salmonella typhimurium*, *Salmonella typhisuis*, and the Ileal symbiont *intracellularis*, as well as the nematode *Trichuris suis* can cause primary enteric disease in the colon of weaned and growing pigs (Harris, 1983; Tubbs, 1987; McOrist and Lawson, 1989; Gebhart et al., 1993). In addition, *Serpulina hyodysenteriae*, *Salmonella* spp., *Campylobacter* spp., and *Escherichia coli* have been associated with invasion secondary to a primary agent (Beer and Rutter, 1972; Wilson, 1986; Tubbs, 1987; Gebhart et al., 1993). *Trichuris suis*, swine whipworm, resides in the cecum and proximal colon and has been implicated as a cause for bacterial proliferation in the colon (Rutter and Beer, 1975). Overlap in clinical manifestations and pathophysiology casts doubt on the primacy of any one of these agents in the pathogenesis of mucohemorrhagic diarrhea in pigs. Most likely, these agents interact and synergize to produce the severe disease observed in the field (Tubbs, 1987). Currently, the mechanisms by which either primary or secondary enteric bacterial pathogens initiate the infectious process and escape regulation by the host are under close scrutiny because of links to human food safety.

Despite extensive study of the etiologic agents of mucohemorrhagic diarrhea in the pig, the specific effect of *T. suis* on the colonic mucosa and on commensal pathogens has not been clearly defined. Experimental infections of specific-pathogen-free pigs and gnotobiotic pigs with individual agents implicated in colonic mucohemorrhagic diarrhea including *T. suis* have generally failed to reproduce the naturally occurring disease (Rutter and Beer, 1975; Hall et al., 1976), except in colostrum-deprived neonates (Babaknani et al., 1993). Experimental infection of pigs with *T. suis* alone succeeds in producing severe disease only if an exceedingly high infective dose of eggs is used (Beer and Rutter, 1972; Batte et al., 1977). However, pigs naturally exposed to *T. suis* on a contaminated dirt lot consistently express severe necrotic proliferative colitis, and mucopurulent lesions in the distal colon away from the site of adult worm attachment (Mansfield and Urban, unpublished results). In contrast, pigs exposed to a drug-abbreviated infection with *T. suis* or immunized with excretory/secretory products derived from cultured adult worms had both reduced worm burdens and pathology of the distal colon following re-exposure to *T. suis* on a contaminated dirt lot (Hill and Urban, 1993). It follows that limiting the growth of pathogenic bacteria resident in the colon by antibiotic therapy should also ameliorate the pathology associated with whipworm

infection. The following study describes a porcine model of mucohemorrhagic diarrhea initiated by subclinical *T. suis* infection, linked to the overgrowth of opportunistic bacteria, and relieved by antibiotic therapy.

2. Materials and methods

2.1. Animals

Outbred Yorkshire cross pigs were used for experimental infections. Pigs were farrowed and reared in confinement on concrete floor pens under conditions that preclude extraneous helminth infections. Pigs were weaned at 6 weeks of age and then 2 weeks elapsed before they were either inoculated with *T. suis* eggs or kept as uninoculated controls. All groups of experimental pigs were housed in confinement facilities under identical management conditions. Pigs were fed a complete pelleted feed (Kool Pig, Southern States, Baltimore, Maryland) twice a day beginning at 3 weeks of age, and water was provided ad libitum.

2.2. Parasites

Adult female *T. suis* were originally isolated from the colon of pigs naturally infected on a contaminated dirt lot. Embryonated eggs were obtained by first culturing adult worms in vitro (Hill et al., 1993), and placing the excreted eggs, separated from the culture media by centrifugation, into potassium dichromate at 22°C for 6 weeks with bubbling. For experimental infection of pigs, eggs were washed twice in sterile water using centrifugation at 1200 *g* for 10 min, and eggs containing viable first stage larvae were counted, resuspended in the desired amount of water and used to inoculate pigs.

2.3. Parasite infections

In several trial experiments, embryonated *T. suis* eggs were given at several doses to determine the dose required to most closely mimic a natural subclinical infection. Pigs in Experiments 1 and 2 were inoculated orally via gavage with 2500 embryonated eggs (which represents a dose of approximately 150 eggs kg^{-1} body weight) based on these trials. Pigs in Experiment 3 were inoculated with graded doses of eggs at 50, 150, and 450 eggs kg^{-1} body weight.

2.4. Experimental design

2.4.1. Experiments 1 and 2

Pigs were divided into 4 treatment groups in a 2×2 matrix square design (Table 1). Five pigs per group were used in Experiment 1, and 8 pigs per group were used in Experiment 2. In Experiment 1, antibiotic treated pigs received erythromycin 22 mg kg^{-1}, i.m., once a day, while in Experiment 2, antibiotic treated pigs received lincomycin/spectinomycin 11 mg kg^{-1}, p.o., once a day. Antibiotics were chosen to

Table 1
Experimental design for Experiments 1 and 2

1	Uninoculated/antibiotic treatment
	No *T. suis* inoculation and given a broad spectrum antibiotic
2	Uninoculated/no antibiotic treatment
	No *T. suis* inoculation and no antibiotic treatment
3	*T. suis* inoculated/antibiotic treatment
	Inoculated with *T. suis* and given a broad spectrum antibiotic
4	*T. suis* inoculated/no antibiotic treatment
	Inoculated with *T. suis* and no antibiotic treatment

control those bacteria generally associated with secondary gastrointestinal infections in *T. suis*-infected pigs. Erythromycin was used in Experiment 1 since *Campylobacter* spp. were isolated from submucosal tissues of pigs infected with *T. suis* in an earlier study. A broader spectrum antibiotic, lincomycin/spectinomycin was chosen for Experiment 2 because it has activity against the major enteric bacteria implicated in mucohemorrhagic diarrhea in swine (Ward and Winkelman, 1990). In these experiments *T. suis*-infected pigs received a single inoculum of 2500 embryonated eggs orally. Pigs were weighed and examined weekly for clinical signs of disease and 10 ml of blood was taken via syringe for complete blood counts. At 45 days after inoculation, all pigs were bled, killed with a captured bolt gun followed by exsanguination. The gastrointestinal tract was examined grossly for pathologic lesions, bacterial isolates made from colonic lymphoglandular complexes (LGCs), and the tissues fixed for histopathology.

2.4.2. Experiment 3

Pigs were inoculated with increasing doses of embryonated eggs of *T. suis* to establish a relationship between egg dose and subsequent lesions. Four groups of 7 eight-week-old pigs were inoculated orally with 0, 50, 150, or 450 eggs kg^{-1} body weight. Pigs were housed in separate pens by group in the same confinement building and examined weekly by physical examination, complete blood counting and weight determination.

2.5. Physical examination and clinical pathology

Animals were weighed and scored for physical parameters (Blood et al., 1979), including temperature, pulse, respiratory rate, mucus membrane color, capillary refill time, skin turgor, lymph node status, status of the skin and hair coat, lung sounds, abdominal palpation, evaluation of appetite, evaluation of diarrhea (classification as to quality, quantity, and type) and evaluation of cardiac function. Blood was aseptically drawn from the vena cava, citrated in a sterile syringe, and evaluated using a Coulter counter and hemoglobinometer, calibrated to provide hematocrit, number of red blood cells, mean corpuscular volume, and hemoglobin. The number of eosinophils, neutrophils, lymphocytes and neutrophilic bands was determined by differential counts from thin blood smears which were fixed in methanol and stained with Wright's stain prior to examination by light microscopy.

2.6. Necropsy procedure

Complete gross necropsies were done excluding examination of the CNS. The entire gastrointestinal tract was removed, divided into small intestines, caecum, and large intestines, slit open, and contents and walls examined for adult *T. suis* and lesions. After lesions were evaluated and samples taken for bacteriologic culture, the colon contents were washed through 0.5 mm screens to retain *T. suis* for counting. Adult whipworm in the contents and in each portion of the colon (proximal and distal) were enumerated. In Experiment 1, 1 g samples of colonic contents were diluted with sterile water and observed using dark field microscopy for the presence of *S. hyodysenteriae*.

A pathologic staging system was developed to score and rank the severity of the lesions observed in the colon. The stages were organized as follows:

Stage 1. Healthy colon unaffected by parasites or bacteria, gut is smooth and tan, 0.50–1.50 mm in thickness, and lymphoglandular complexes (LGCs) contain follicles made up of lymphocytes and macrophages with normal entrapped mucosal glandular crypts.

Stage 2. Colon has patchy areas of hemorrhage ($< 50\%$ of the colon is hemorrhagic), is 1.51–2.50 mm in thickness, is slightly roughened and LGCs have mild to moderate infiltrates of inflammatory cells including eosinophils.

Stage 3. Colon is hemorrhagic ($> 50\%$ of its surface), 2.50 mm or greater in thickness, is markedly roughened and LGCs appear with mucopurulent debris within the entrapped glandular crypts.

Stage 4. Colon is covered with a fibrinonecrotic pseudomembrane, is 2.50 mm or greater in thickness, may or may not have denuded areas where the mucosa is sloughed and LGCs, if not obliterated by pseudomembranes, are filled with mucopurulent debris.

2.7. Histopathology

Full thickness tissue samples were taken from the caecum, proximal colon, mid colon and distal colon for histopathologic evaluation. Sections were also taken from worm attachment sites and LGCs. All samples were fixed in 10% formalin, embedded in paraffin, sectioned at 5 m thickness and stained with both hematoxylin and eosin and Warthin–Starry silver stains. Sections were observed and photographed on an Olympus BH-2 microscope from $10\times$ to $100\times$ magnification.

2.8. Bacteriologic sampling

The LGCs were sampled for bacteria during the necropsy examination. The colon was slit longitudinally, the contents removed to a separate bucket, and the surface washed with water until intestinal contents were removed. After scoring the tissues for gross pathologic changes, the LGCs were seared with a hot spatula until the superficial mucosa was charred. A sample was taken through the seared area using a sterile swab. The swab was placed in transport media (Port-A-Cul Transport Tubes, Fisher Scientific, King of Prussia, PA) and delivered to the diagnostic microbiology laboratory (University of Maryland, College Park, MD) where it was plated on media for isolation of selected

members of the Enterobacteriaceae, including *Campylobacter* spp., *Salmonella* spp., and *Escherichia coli*. Samples from infected pigs were inoculated onto defined media (Prescott and Chirino-Trejo, 1986), grown at a range of temperatures and tested for Gram staining and biochemical attributes to determine species. Culturing was targeted toward bacterial species that have been linked to mucohemorrhagic colitis in pigs.

2.9. Statistics

The size and number of lymphoglandular complexes and the clinical pathology values from the four treatment groups were compared using the Kruskal–Wallis rank sums test. All values are given as the mean ± the standard deviation.

3. Results

3.1. Clinical signs

Only pigs in Group 4 in Experiments 1 and 2 showed clinical signs of disease during the course of the study. These animals were judged normal until 14 days after infection at which time they all developed a liquid brown diarrhea and a rough hair coat. The diarrhea from some pigs had small amounts of fresh blood. One pig in Group 4, Experiment 1, was weak and anorexic, but survived until the conclusion of the experiment.

3.2. Clinical pathology

Pigs in Groups 1, 2 and 3 showed no abnormal clinical pathological values during the course of the study. Pigs in Group 4 had a significant decrease in hematocrit and a significant increase in band neutrophils when compared with pigs in Groups 1, 2 and 3 (Table 2). Circulating eosinophils were increased in some pigs in Group 4, but this increase was not significant when the group values were compared to those of Groups 1, 2 and 3 (Table 2). Pigs in Experiment 3 showed similar changes in hematocrit and band

Table 2
Abnormal clinical pathology values of pigs in Experiments 1 and 2. Values represent the mean ± the standard deviation

Group	Treatment	Hematocrit	Band neutrophils	Eosinophils
1	No *Trichuris*; antibiotic treated	40.7 ± 1.5^{a}	8 ± 8^{c}	17 ± 8
2	No *Trichuris*; no antibiotics	41.9 ± 3.3^{a}	13 ± 14^{c}	28 ± 14
3	*Trichuris* infected; antibiotic treated	39.4 ± 7.4^{ab}	31 ± 15^{c}	31 ± 14
4	*Trichuris* infected; no antibiotics	33.9 ± 1.7^{b}	58 ± 27^{d}	38 ± 22
	Reference values	(36–43)	(0–4)	(0–15)

Values represent mean ± standard deviation.
Values with different letters were significantly different ($P < 0.05$) using Kruskal–Wallis rank sums test.
Results of Experiments 1 and 2 were combined.

Table 3
Number of *Trichuris suis* recovered from proximal and distal colon and colon contents at necropsy in Experiments 1 and 2

Group	Treatment	Number of *Trichuris* recovered			
		Proximal colon	Distal colon	Contents	Total
1	No *Trichuris* antibiotic treated	0	0	0	0
2	No *Trichuris*; no antibiotics	0	0	0	0
3	*Trichuris* infected; antibiotic treated	295 ± 218	7 ± 11	35 ± 22	336 ± 232 (range 93–832)
4	*Trichuris* infected; no antibiotics	371 ± 87	15 ± 21	38 ± 50	423 ± 52 (range 342–481)

Values represent mean ± standard deviation.
Results of Experiments 1 and 2 were combined.

neutrophils at the highest *T. suis* dose rate (450 eggs kg^{-1}) while pigs in all other groups had values within normal ranges for the species (data not shown).

3.3. Gross pathology

Gross lesions were observed only in pigs from Group 4, and in pigs from the highest inoculation dose (450 eggs kg^{-1} body weight) in Experiment 3. All organ systems observed in all *T. suis* inoculated pigs, excluding the gastrointestinal tract (GI), were judged to be normal when compared with uninfected control pigs. The majority of adult worms was found in the cecum and proximal colon in all experiments and uninfected control pigs were free of *T. suis* or other nematode infections (Table 3).

All pigs in Groups 1 and 2 were judged to have Stage 1 pathology with normal colons, while pigs in Group 3 were judged to have Stage 2 pathology. In Experiment 1, one pig in Group 4 had Stage 2 pathology, while two pigs had Stage 3, and two pigs had Stage 4 pathology of the colon. In Experiment 2, five pigs in Group 4 had Stage 2 pathology, while two pigs had Stage 3, and one pig had Stage 4 pathology of the colon.

Changes in the GI tract of pigs in Group 4 were divided into those occurring directly at the site of worm attachment in the caecum and proximal colon, and those occurring in the distal colon. In Experiments 1 and 2, the 3 pigs in Group 4 with severe clinical signs were the ones with Stage 4 pathology with necrotic pseudomembranes and discrete patches where mucosa had been sloughed in the proximal colon. Hemorrhage of the mucosa was confined to the proximal colon in all pigs with *T. suis* and was quantitatively and quantitatively more severe in pigs from Group 4 than pigs from Group 3 in both experiments. Pigs from both Group 3 and Group 4 had an increase in thickness of the proximal colon when compared with pigs in Groups 1 and 2, but thickening of the gut was significantly greater in pigs in Group 4 (Group 3, 1.75 ± 0.35 mm vs. Group 4, 2.52 ± 0.41 mm). Gut thickness measured in pigs from Group 1 (0.92 ± 0.14 mm) and Group 2 (0.98 ± 0.13 mm) was judged to be normal. Pigs with Stage 3 or 4 pathology

Fig. 1. Cross section of proximal colon from a group 4 pig, *Trichuris suis* inoculated and untreated with antibiotics, 45 days after inoculation. Colonic tissue was fixed in formalin, stained with hematoxylin and eosin and observed at magnification × 40. Section shows destruction of surface epithelial cells in close proximity to adult *Trichuris* (arrow).

had a rough appearance to the proximal colon in the site of worm attachment. This correlated to histopathologic evidence of destruction of surface epithelial cells (Fig. 1).

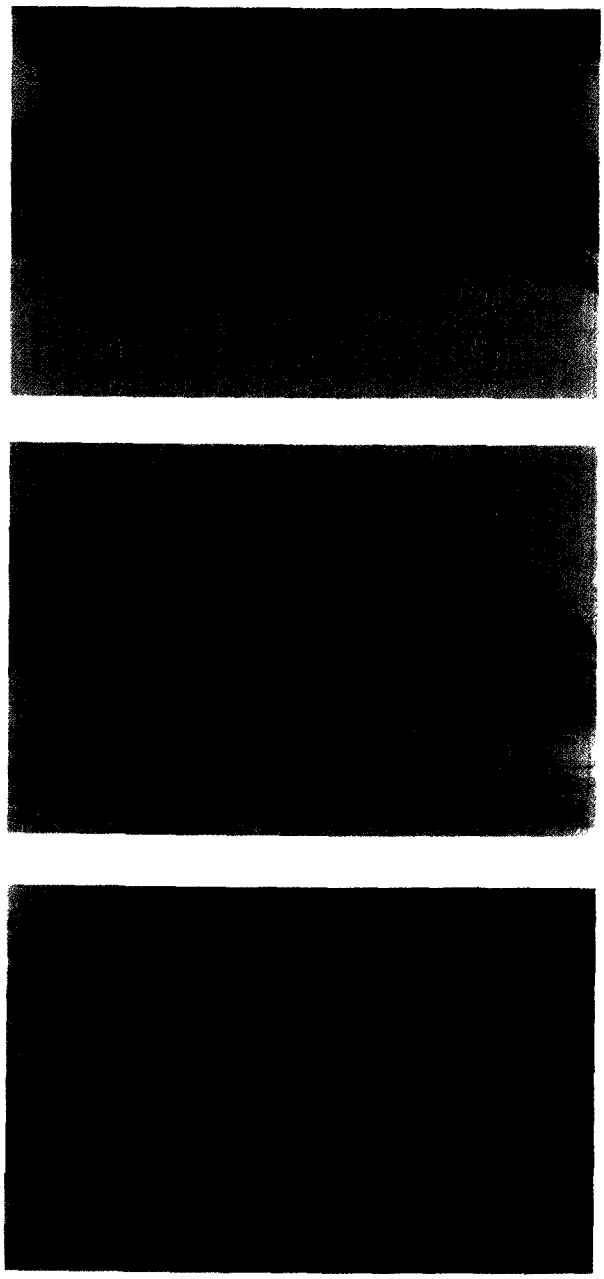
In the distal colon of pigs from Group 4 there was an enlargement of the LGCs. They appeared as raised nodular bulls-eye lesions with mucopurulent abscesses. The size of the lesion depended on the size of the lymphoid nodule, the degree of inflammatory cell infiltrate and secondary bacterial proliferation, which was confirmed with histopathology (Fig. 2). Some of the LGC lesions in pigs with Stage 3 pathology and all of the LGC lesions in pigs with Stage 4 pathology were inflamed.

3.4. Histopathology

Microscopic lesions were found only in *T. suis* infected pigs. Lesions in pigs from Group 4 were more severe than those in pigs from Group 3 and were distinctly different in the proximal and distal colon. There was thickening of the muscularis and mucosa at

Fig. 2. Lymphoglandular complexes in the colon of pigs from Experiment 2, hematoxylin and eosin stain, magnification × 10. (A) Upper panel shows a lymphoglandular complex from a normal uninfected control pig from Group 1. Notice that the lymphoid tissue lies beneath the muscularis mucosa and that Crypts of Lieberkuhn extend into and are surrounded by the lymphoid follicle. (B) Middle panel shows a lymphoglandular complex from a *Trichuris suis* infected pig from Group 3 that received broad spectrum antibiotics. There is a thickening of the muscularis and mucosal layers of the colon and a generalized increase in the number of cells in the lymphoid follicle in response to infection with the parasite. The arrow indicates the presence of germinal centers in this Group 3 pig. (C) Lower panel shows a lymphoglandular complex from a *Trichuris suis* infected pig from Group 4 that was not treated with antibiotics. Notice the large size, the central abscess with neutrophilic debris, the epithelial membrane bounding the abscess and the compressed lymphoid follicle surrounding the abscess.

the site of worm attachment in the proximal colon (Fig. 3), destruction of the absorptive cells on the surface of the colon (Fig. 1), crypt destruction with loss of goblet cells (Fig. 1) and an increase in inflammatory cells in the lamina propria (Fig. 1). Pigs from Group



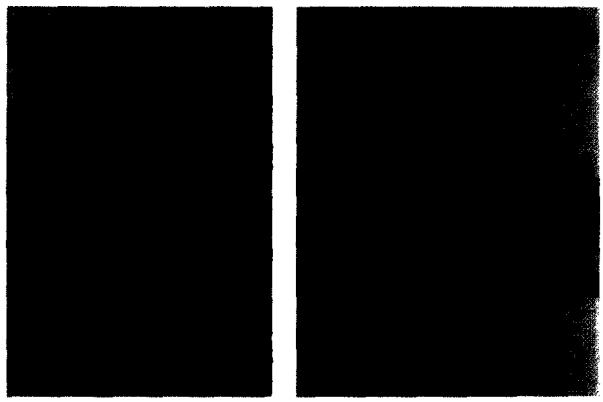


Fig. 3. Cross section of proximal colon from *Trichuris suis* inoculated and antibiotic untreated pigs (Group 4) (*left*) compared with that from a noninfected, untreated pig (Group 2) (*right*) 45 days after inoculation. Colonic tissue was fixed in formalin, stained with hematoxylin and eosin and observed at magnification × 10.

3 had an increase in the number of lymphocytes and macrophages in the lamina propria compared with pigs in Groups 1 and 2. Pigs in Group 4 had an increase in lymphocytes, macrophages, neutrophils, eosinophils and plasma cells in the lamina propria and an increase in bacteria in the crypts of Lieberkuhn causing them to be distended throughout the colon. Using a Warthin–Starry stain, masses of bacteria were visualized in close proximity to adult worms in pigs from Group 4, but not in pigs from Group 3 (Fig. 4). In both Experiment 1 and 2, at least one pig from Group 4 had porcine intestinal adenomatosis lesions (Fig. 5(A)). Affected crypts had many immature enterocytes with numerous mitotic figures and a complete loss of goblet cells. Intracellular bacteria within the apical cytoplasm of these enterocytes were demonstrated to be the Ileal symbiont *intracellularis* using Warthin–Starry staining (Fig. 5(B)).

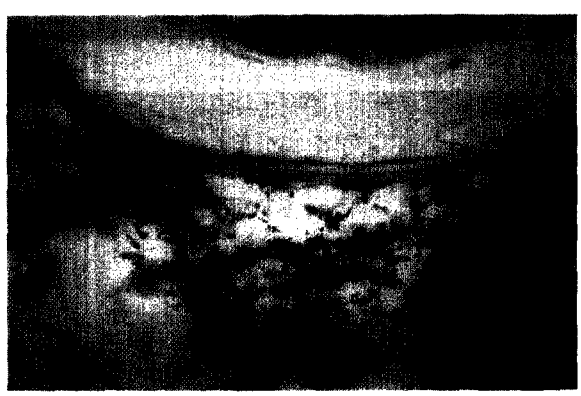


Fig. 4. Bacteria proliferating in close proximity to adult *Trichuris suis*, magnification × 100. Tissue section was taken from proximal colon, fixed in formalin and stained with Warthin–Starry silver stain to demonstrate bacteria. The solid arrow indicates an adult worm in cross section.

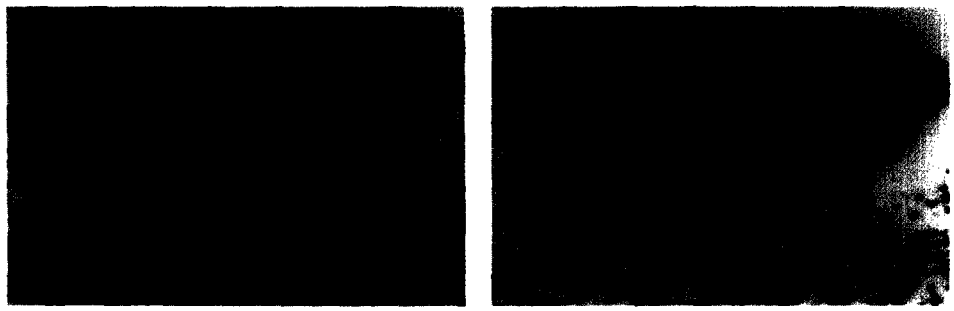


Fig. 5. Crypt of Lieberkuhn from the proximal colonic mucosa of a pig infected with *Trichuris suis* which did not receive antibiotic treatment, magnification × 40. (A) (left) Section stained with hematoxylin and eosin shows the loss of goblet cells, epithelial cell hyperplasia, immature epithelial cells with many prominent mitotic figures (*arrows*) that are characteristic of porcine intestinal adenomatosis. (B) (right) Contiguous serial section stained with Warthin–Starry stain which shows intracellular bacteria in the apical cytoplasm of the PIA affected cells. Arrow points to bacteria identified as the Ileal symbiont *intracellularis*.

In the distal colon, away from the site of adult worm attachment, there was an increase in the size of LGCs in the submucosa of pigs in Groups 3 and 4 (Table 4). (Fig. 2). The LGCs were significantly larger in pigs from Group 4 than in pigs from Group 3 ($P \leq 0.05$), and the cellular composition of the lymphoid tissue within the nodule was also different between the two groups. The LGCs from pigs in Group 3 had an increase in the number of lymphocytes and macrophages compared with normal nodules of pigs in Groups 1 and 2. A few eosinophils were observed around the periphery of these nodules. The LGCs from pigs in Group 4 had an increase in lymphocytes and macrophages, but many neutrophils were also observed within the nodules. Some of the macrophages appeared to be undergoing apoptosis. There was also a large increase in eosinophils in the periphery of the nodules and a few eosinophils among the lymphoid follicle. Two of five pigs from Group 3 in Experiment 1 and no pigs from Group 3 in Experiment 2 had bacteria in the LGC crypts. Three of five pigs from Group 4 in Experiment 1, and seven of eight pigs from Group 4 in Experiment 2 had bacteria within the epithelial crypts entrapped in the LGCs. In these LGCs, bacteria and mucopurulent

Table 4
Size and number of lymphoglandular complexes in the colon of pigs in four treatment groups in Experiments 1 and 2

	Group Treatment	Lymphoglandular complexes	
		Number	Size
1	No *Trichuris*; antibiotic treated	288 ± 495	0.25 ± 0 [a]
2	No *Trichuris*; no antibiotics	1313 ± 347	0.31 ± 0.11 [b]
3	*Trichuris* infected; antibiotic treated	350 ± 337	0.40 ± 0.10 [c]
4	*Trichuris* infected; no antibiotics	738 ± 663	0.63 ± 0.10 [d]

Values represent mean ± standard deviation.
Values with different letters were significantly different ($P < 0.05$) using Kruskal–Wallis rank sums test.
Results of Experiments 1 and 2 were combined.

Fig. 6. Lymphoglandular complex from a *Trichuris suis* inoculated, nonantibiotic treated pig 45 days after inoculation, magnification × 40. Solid arrow indicates the specialized follicle associated epithelial membrane lining the entrapped crypt of the LGC. The open arrow indicates mucopurulent cellular and bacterial debris forming a crypt abscess within the LGC.

cellular debris destroyed the architecture of the LGC follicle and compressed the lymphoid material to a small area in the submucosa (Fig. 2(C)). Serial sections of these crypt abscesses showed bacteria in connective tissue and muscle below the LGC. None of the LGCs that were sectioned had *T. suis* adults or larvae within the nodule. Histology confirmed the presence of LGCs in all uninoculated, untreated control pigs (Fig. 2). In pigs from Group 3 with expanded lymphoid follicles in the LGCs in response to *T. suis* infection, sections of the enlarged LGCs showed a specialized epithelial membrane lining the entrapped crypts overlying the lymphoid cells of the follicle, which suggests a role as an antigen-processing structure (Fig. 6).

3.5. Bacterial isolation

All pigs from Group 4 had at least 3 isolates of bacteria. These included *Campylobacter coli, C. jejuni, C. lari, Campylobacter spp.* (unidentified species), *Escherichia coli* (strain unidentified), *Escherichia fergusonii, Enterobacter intermedium, Enterobacter cloacae* and *Pseudomonas fluorescens*. No *Salmonella* were isolated. Ileal symbiont *intracellularis* bacteria were demonstrated by Warthin–Starry staining in epithelial cells in the mucosa and LGCs in one of five pigs from Group 4 in Experiment 1, and four of eight pigs from Group 4 in Experiment 2. No bacteria were isolated from the LGCs of uninoculated pigs in Groups 1 and 2. Bacteria were isolated from abscessed LGCs of 2 pigs from Group 3 in Experiment 1, while pigs from Group 3 in Experiment 2 had no culturable bacteria. No *Campylobacter spp.* were isolated from pigs in Group 3 in either experiment. No *S. hyodysenteriae* were seen in dark fields preparations of colon contents of pigs from Experiment 1. Additionally, *S. hyodysenteriae* was not observed in tissue sections from pigs in any experiment at a higher density than five organisms per high powered field, the threshold for designation of this organism as a etiologic agent of disease (Tubbs, 1987).

Table 5
Size and number of lymphoglandular complexes in the colon of three groups of pigs inoculated with increasing dose of embryonated *Trichuris suis* eggs, and a group of control uninfected pigs from Experiment 3

Group	Treatment	Lymphoglandular complexes	
		Number	Size
1	Uninfected	550 ± 531	0.26 ± 0.04 [a]
2	Low dose; 50 eggs kg^{-1}	765 ± 677	0.28 ± 0.10 [a]
3	Medium dose; 150 eggs kg^{-1}	483 ± 292	0.29 ± 0.06 [a]
4	High dose; 450 eggs kg^{-1}	625 ± 250	0.39 ± 0.07 [b]

Values represent mean ± standard deviation.
Values with different letters were significantly different ($P < 0.05$) using Kruskal–Wallis rank sums test.

3.6. Experiment 3

The number of adult *T. suis* that established in the caecum and proximal colon of pigs from Experiment 3 was lower than in Experiments 1 and 2 where an inocula of approximately 150 eggs kg^{-1} body weight was used. Recoveries of adult *T. suis* in Experiment 3 were: Uninoculated control group = 0; low dose group (50 eggs kg^{-1}) = 5.4 ± 5.0; medium dose (150 eggs kg^{-1}) 35.0 ± 16; high dose (450 eggs kg^{-1}) = 661 ± 133. However, similar pathologic changes were observed in pigs given the highest dose of *T. suis* as those seen in pigs from Group 4 in Experiments 1 and 2. Pigs in the high dose group had liquid brown diarrhea that began about 14 days after infection. The hematocrit was the only clinical pathologic value that was abnormal in the high dose group. As with pigs in Group 4 in Experiments 1 and 2, the hematocrit was decreased by 10 points in the high dose group.

The larger the dose of embryonated eggs given to weaned pigs the greater the effects on the colonic mucosa. Pigs in the high dose group were judged to have Stage 3 and 4 pathology while those in the uninoculated control group had Stage 1, and those in the lower dose groups (50 and 150 eggs kg^{-1}) had Stage 2. Pathology in the high dose group was similar to that seen in pigs in Group 4 of Experiments 1 and 2. There was diffuse hemorrhage, increased thickness, and roughness of the proximal colon, with pseudonecrotic membranes in two high dose pigs. In the distal colon of high dose pigs there were enlarged LGCs that had crypt abscesses (Table 5).

4. Discussion

We have minimized the effect of secondary bacterial infection associated with *T. suis* induced pathology in the colon of growing pigs through the strategic application of broad spectrum antibiotics. Severe mucohemorrhagic pathology was grossly detectable in the colon and clinical pathologic changes were observed in pigs experimentally inoculated with *T. suis* eggs and maintained in confinement on concrete floor pens, while pigs similarly maintained, but not inoculated with *T. suis*, had no observable disease or lesions. Likewise, pigs inoculated with and treated with broad spectrum antibiotics to prevent bacterial proliferation in the gut had no *T. suis* clinical signs, no

gross pathologic lesions and only mild histopathologic changes in the lamina propria directly surrounding adult worms. These results suggest that infection with *T. suis* predisposes young pigs to colonic disease by allowing bacteria to invade and proliferate and shows that bacteria are required to induce the severe pathology observed in *T. suis* infected pigs. Additionally, removal of bacteria in *T. suis* infected pigs by antibiotic treatment prevented pathology in the colon except in the area immediately surrounding adult worms. This suggests that this nematode may act on distant sites through production of secreted products or through a modulation of the host inflammatory or immune response to invading bacteria. This observation is the reciprocal to the vaccination of pigs with a drug-abbreviated *T. suis* infection or with parasite derived antigens that results in not only reduced worm recoveries, but reduced pathology following natural exposure to *T. suis* eggs on a contaminated dirt lot (Hill and Urban, 1993).

Mild to moderate colonic mucohemorrhagic diarrhea with secondary bacterial proliferation in the lumen, mucosa and lymphoglandular complexes is induced in young pigs by low level experimental infections with *T. suis*. This occurs despite strict isolation in concrete confinement housing which decreases the amount of pathogens ingested by pigs during rooting behavior. Pigs with *T. suis* had pathologic changes in the colon in the site of worm attachment but also had changes in sites distant to the worms. There were a range of pathologic manifestations in *T. suis* infected pigs. All *T. suis* infected pigs had thickening of the muscularis and mucosa throughout the colon, increase in inflammatory cells in the lamina propria and destruction of surface epithelial cells at the site of worm attachment. Non-antibiotic treated pigs had more severe pathology. The degree of pathologic change was increased in non-antibiotic treated pigs at the site of adult worm attachment as well as at sites distant to worm attachment in the distal portion of the colon. The mucosa and muscularis were thicker, larger portions of the proximal colon were hemorrhagic, and pseudonecrotic membranes were seen in these pigs. In the distal colon, the most prominent change was an increase in the size of the LGCs. The LGCs were enlarged due to an increase in the inflammatory cells in the LGC nodule and the proliferation of bacteria in the entrapped mucosal crypts which often resulted in crypt abscessation with purulent debris. Serial sections of these lesions identified bacteria in the submucosa and muscularis suggesting the LGC as a route for invasion and dissemination of pathogenic organisms. These pathologic changes and bacterial overgrowth were ameliorated by treatment with two classes of broad spectrum antibiotics.

Clinical signs of disease and pathology observed in the colon were related to the dosage of *T. suis* eggs inoculated into pigs. Only the pigs receiving the highest dose of embryonated eggs of *T. suis* (450 eggs kg^{-1}, with a resulting adult worm burden of 661 ± 133) had severe signs and pathology. This correlated with results of Experiments 1 and 2 where pigs with the most severe pathology had established worm burdens of 423 ± 52 *T. suis* adults. These results prove that extremely high doses of *T. suis* eggs are not required to produce severe pathology (Beer and Rutter, 1972) and suggest that the bacterial component of the infection is very important in determining the severity of the outcome. Others have observed the relationship between *T. suis* infection in young pigs and the production of severe secondary bacterial infection of the colon with subsequent pathological changes (Rutter and Beer, 1975). Experimental infections of

specific pathogen free and gnotobiotic pigs with *T. suis* were unsuccessful in producing either clinical signs or disease.

Lymphoglandular complexes have been described in several mammalian species using light and electron microscopy to describe the structure (Biswal et al., 1953; Liebler et al., 1988a,b; Morfitt and Pohlenz, 1989; Parsons et al., 1991). These structures are characterized by a differentiated epithelium that is associated with a lymphoid follicle. This epithelium is capable of nondegradative transport of immunogenic materials from the lumen of the gut to the lymphoid follicle by means of specialized cells termed follicle associated epithelial cells or M cells. This epithelium lines the entrapped crypts of the LGCs and is contiguous with the mucosal surface through a minute pore, giving the LGC the appearance of a bulls-eye nodule on the mucosal surface of the colon. Reported investigations of colonic gut associated lymphoid tissues (GALT) are scarce and functional proof of LGCs as an antigen processing organ is lacking. Photographs of enlarged cystic LGCs have been published in swine with dysentery from different causes including *T. suis* and bacteria (Batte and Moncol, 1972; Ferguson et al., 1980), but these authors described the enlargement without identifying the structure as a lymphoid follicle and without ascribing a cause for the enlargement. We have identified LGCs in the colon of *T. suis* infected pigs and demonstrated that they are enlarged as a result of a combined infection with *T. suis* and colonic bacteria. In fact, bacteria are found in large numbers within entrapped glands and occasionally within entrapped enterocytes in the crypts of the LGCs in pigs infected with *T. suis*, whereas normal uninfected pigs have no bacteria in these structures. Additionally, serial sections of LGCs with crypt abscesses showed that bacteria had invaded into the submucosa and muscularis deep to the LGC providing a route to a generalized bacteremia.

These studies show that concurrent infections with *T. suis* enhances the ability of opportunistic bacteria to multiply and cause disease and pathology. This may have implications for human food safety concerns (Anonymous, 1993), in that, many of the bacteria cultured from the LGCs in the colon of infected pigs are pathogenic for humans (Butzler, 1984). Proliferation of these bacteria secondary to a nematode infection may be caused by a more generalized phenomenon than simple invasion of bacteria into parasite damaged tissues. The fact that infection with *T. suis* can predispose distant sites in the colon to bacterial invasion suggests a more general mechanism where a strong antiworm response that elicits a pattern of cytokine synthesis characteristic of the T-helper cell Th2 phenotype can dampen the protective response to microbial infections (Urban et al., 1992). In fact, several different GI nematode infections have been shown to induce strong Th2 responses in the GALT where the synthesis of interleukins 4 and 10 are increased and interferon-gamma, which is critical to the immune response to many intracellular pathogens, is diminished (Svetic et al., 1993; Else et al., 1994). Given that exogenous interleukin-12 (Finkelman et al., 1994) and both endogenous and exogenous increases in interferon-gamma (Urban et al., 1993) can convert worm induced Th2 responses in gut associated lymphoid tissue and suppress immunity to the worm, it is conceivable that a strong antiworm response could downregulate immunity to microbial infections. The induction of mucohemorrhagic diarrhea and associated colonic pathology by subclinical *T. suis* infection and secondary bacterial infection in swine would appear to be an excellent model to test this hypothesis.

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